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Aspicilia volcanica, a new saxicolous lichen from Northeast China

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Abstract — *Aspicilia volcanica* from Heilongjiang in Northeast China is described as new to science. The species is characterized by the thin grayish olive thallus, moniliform branched anastomosing paraphyses, and the presence of stictic and hypostictic acids. It grows on volcanic rock in temperate region. ITS nrDNA sequence analysis supports the taxonomic distinctness of this species.

Key words — Asia, biodiversity, *Megasporaceae*, *Pertusariales*, taxonomy

Introduction

The lichen-forming ascomycete genus *Aspicilia* A. Massal. (*Pertusariales*, *Megasporaceae*) displays a considerable range of morphological variation (Sohrabi et al. 2011). Within the genus a number of species have radiating thalli and elongate, often ± diverging and ± branched marginal areoles, closely attached to the substratum (Nordin et al. 2011). *Aspicilia* s. lat (which in the traditional sense includes *Cicinaria*, *Lobothallia*, *Sagedia,* and *Teuvoa*) has a worldwide distribution and covers a diverse assemblage of 200–250 species (Kirk et al. 2010, Sohrabi et al. 2013). Forty-four species of *Aspicilia* s. str. (which includes *Teuvoa*) have been previously been reported from China (Wei 1991, Abbas & Wu 1998, Sohrabi et al. 2010, Li et al. 2013), but only two *Aspicilia* species (*A. subdepressa, A. transbaicalica*) were reported from Northeast China (Wei 1991, Li et al. 2013).

During a study of the lichen genus *Aspicilia* in China, some interesting specimens were collected from the Wudalianchi volcanic mountains, Heilongjiang, Northeast China. Careful morphological and anatomical examination combined with chemical and nrITS sequence analyses has confirmed one representative, here described *Aspicilia volcanica*, as new to science.

Materials & methods

Specimens were collected from the Wudalianchi Scenic Area, Wudalianchi City, Heilongjiang Province, China, and are preserved in Lichens Research Center in Arid Zones of Northwest China, Xinjiang University, Urumqi, Xinjiang, China (XJU). The lichen specimens were examined morphologically using a Leica Zoom 2000 dissecting microscope and anatomically with an Olympus CH compound microscope. Sections, mainly cut by hand, were studied in water. Chemical constituents were identified by thin-layer chromatography using solvent systems A, B, and C (Orange et al. 2010). Photos of the thallus and anatomical structures were taken with a Nikon Eclipse E200 stereomicroscope with Canon Digital Camera Powershot A640 and Nikon Digital Camera D50.

DNA EXTRACTION, PCR AMPLIFICATION, & SEQUENCING: DNA was extracted using DNAsecure Plant DNA Kit (Tiangen, China). PCR amplification followed Martín et al. (2003) and the manufacturer's recommendations (Tiangen, China). The whole nrITS region (ITS1-5.8S-ITS2) was targeted using the primers ITS1-F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). Thermocycling protocols were 95°C for 3 min linked to 35 cycles at 94°C for 30 s, 54°C for 30 s, and 72°C for 1 min, with a final extension of 72°C for 10 min. PCR products were screened on 1% agarose gels stained with ethidium bromide and sequenced by the Genewiz Inc. (Beijing).

PHYLOGENETIC ANALYSIS & SEQUENCE COMPARISON: We generated one ITS sequence (KM609324) and aligned it with 34 representatives (including *Lobothallia alphoplaca* as the outgroup) both by ClustalW and Muscle implemented in MEGA 6 (Tamura et al. 2013), then optimized manually. The alignment matrix of 501 nucleotide positions was submitted to TreeBase (S16691).

A phylogeny was inferred by the Maximum Likelihood method based on the Tamura-Nei model in MEGA6 and by using Bayesian inference based on GTR model with rates=Invgamma in MrBayes (Huelsenbeck & Ronquist 2011). For ML analysis, initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.4216)).

Results & Discussion

ITS sequence analysis

The ITS sequence analysis supports *A. volcanica* as a separate species (FIG. 1) within the *A. cinerea*-group with close affinities to the common species, *A. cinerea* (query cover 100%, 93% identity and 2% gap). *Aspicilia volcanica* is also close to *A. laevata* (query cover 91%, 90% identity, 1% gap) and *A. indissimilis* (query cover 92%, 90% identity, 1% gap).

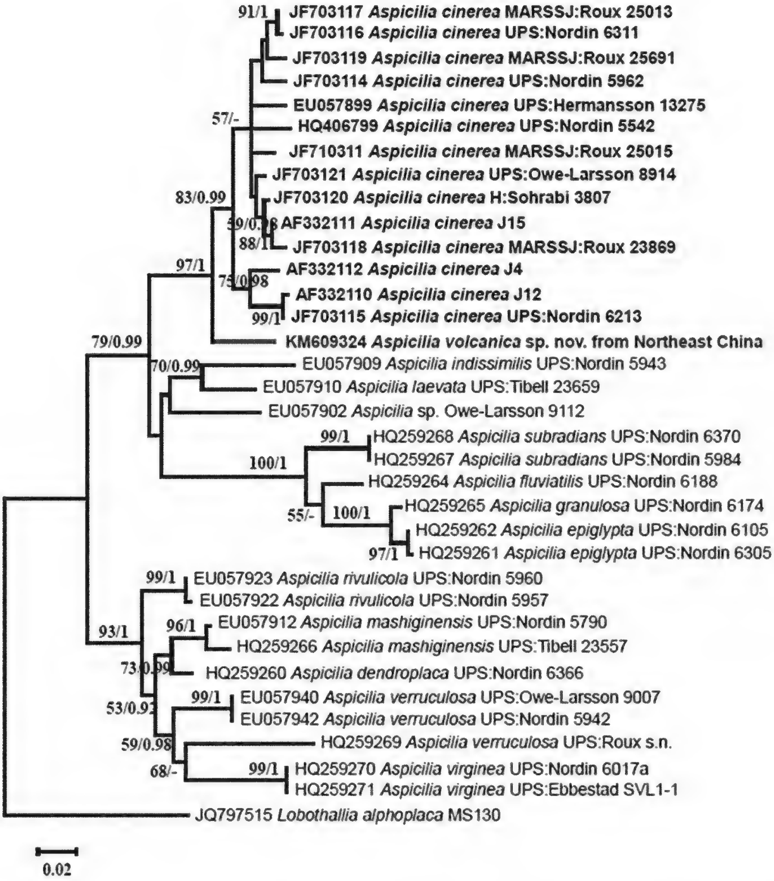
91/1
JF703117 *Aspicilia cinerea* MARSSJ:Roux 25013
JF703116 *Aspicilia cinerea* UPS:Nordin 6311
JF703119 *Aspicilia cinerea* MARSSJ:Roux 25691
JF703114 *Aspicilia cinerea* UPS:Nordin 5962
EU057899 *Aspicilia cinerea* UPS:Hermansson 13275
57/-
HQ406799 *Aspicilia cinerea* UPS:Nordin 5542
JF710311 *Aspicilia cinerea* MARSSJ:Roux 25015
JF703121 *Aspicilia cinerea* UPS:Owe-Larsson 8914
83/0.99
JF703120 *Aspicilia cinerea* H:Sohrabi 3807
59/0.98
AF332111 *Aspicilia cinerea* J15
88/1
JF703118 *Aspicilia cinerea* MARSSJ:Roux 23869
97/1
AF332112 *Aspicilia cinerea* J4
75/0.98
AF332110 *Aspicilia cinerea* J12
99/1
JF703115 *Aspicilia cinerea* UPS:Nordin 6213
79/0.99
KM609324 *Aspicilia volcanica* sp. nov. from Northeast China
70/0.99
EU057909 *Aspicilia indissimilis* UPS:Nordin 5943
EU057910 *Aspicilia laevata* UPS:Tibell 23659
EU057902 *Aspicilia* sp. Owe-Larsson 9112
99/1
HQ259268 *Aspicilia subradians* UPS:Nordin 6370
100/1
HQ259267 *Aspicilia subradians* UPS:Nordin 5984
HQ259264 *Aspicilia fluviatilis* UPS:Nordin 6188
55/-
100/1
HQ259265 *Aspicilia granulosa* UPS:Nordin 6174
HQ259262 *Aspicilia epiglypta* UPS:Nordin 6105
97/1
HQ259261 *Aspicilia epiglypta* UPS:Nordin 6305
99/1
EU057923 *Aspicilia rivulicola* UPS:Nordin 5960
EU057922 *Aspicilia rivulicola* UPS:Nordin 5957
96/1
EU057912 *Aspicilia mashiginensis* UPS:Nordin 5790
93/1
HQ259266 *Aspicilia mashiginensis* UPS:Tibell 23557
73/0.99
HQ259260 *Aspicilia dendroplaca* UPS:Nordin 6366
99/1
EU057940 *Aspicilia verruculosa* UPS:Owe-Larsson 9007
53/0.92
EU057942 *Aspicilia verruculosa* UPS:Nordin 5942
59/0.98
HQ259269 *Aspicilia verruculosa* UPS:Roux s.n.
99/1
HQ259270 *Aspicilia virginea* UPS:Nordin 6017a
68/-
HQ259271 *Aspicilia virginea* UPS:Ebbestad SVL1-1
JQ797515 *Lobothallia alphoplaca* MS130
0.02

FIGURE 1. Position of the new species in the ITS phylogenetic tree of *Aspicilia* with *Lobothallia alphoplaca* as outgroup. ML bootstrap support / Bayesian posterior probabilities are shown at nodes; nodes with ML support <50 and PP <0.90 are collapsed. Branch lengths refer to the number of substitutions per site. Herbarium acronyms (where available) and specimen numbers, and isolate numbers are attached to the sequences downloaded from GenBank.

Taxonomy

Aspicilia volcanica Ismayil, A. Abbas & S.Y. Guo, **sp. nov.** FIGURE 2

FUNGAL NAME FN 570144

Differs from *Aspicilia cinerea* by its thin grayish olive thallus, the presence of stictic and hypostictic acids, and the absence of norstictic acid.

TYPE: China. Heilongjiang: Wudalianchi scenic area, 48°39.321′N 126°09.311′E, alt. 280 m, 15 July 2011, Gulbostan Ismayil & A. Abbas 20111154 (**Holotype**, HMAS-L; **isotype**, XJU; GenBank KM609324).

Etymology: The specific epithet *volcanica* refers to the substrate of the type specimen. There are 14 volcanic mounts in Wudalianchi, Heilongjiang, Northeast China.

Thallus grayish olive to olive, areolate to rimose, thin; margin flat, elongated, branching and diverging, thin, creamy white or whiter than thallus. Areoles irregular to angular and slightly verrucose, flat to ± convex, somewhat ± dispersed; separated by cracks and uneven, 0.2–0.5 mm wide and up to 1.2 mm long. Cortex paraplectenchymatous, 26–39 µm thick, uppermost part greenish black, without crystals, K–, covered with an epinecral layer, 5–10 µm thick. Medulla lax and K–. Hypothallus white or whiter than thallus, smooth at margins. Photobiont chlorococcoid, cells ± round, 8–18 µm in diam.

Apothecia aspicilioid, usually rather common, 1–2 per areole, irregular and sometimes round or angular, 0.3–0.7 mm in diam. disc black, concave, epruinose. Thalline margin flat to elevated in older apothecia, concolorous with or whiter than thallus, 0.1–0.2 mm thick. Epihymenium blackish or deep green, without crystals, K+ pale. Hymenium hyaline, 98–143 µm tall, I+ greenish blue to cupper red, K–. Hypothecium pale, 52–65 µm thick, K–, I+ persistently ink blue. Paraphyses moniliform (uppermost part distinctly moniliform), upper cells globose, branched and anastomosing. Asci *Aspicilia*-type, clavate, 8-spored, 57–104 × 16–31 µm. Ascospores hyaline, simple, ellipsoid, (13–)16–23(–26) × (10–)13–16 µm, with oil drops. Pycnidia not found.

Spot tests —K+ greenish yellow, KC–, C–, I–, PD–, UV–.

Secondary metabolites —Hypostictic acid, connorstictic acid, stictic acid (TLC).

Ecology —*Aspicilia volcanica* grows on black rock from volcanic mountain. Associated species include *Rhizocarpon* sp., *Rhizoplaca* sp. At present, it is only known from the volcanic mountain region in Northeast China at 280–400 m elevations.

Additional specimen examined — CHINA. Heilongjiang: Wudalianchi, Wudalianchi Scenic Area, 48°43.213′N 126°27.220′E, alt. 400 m, 13 Jul. 2011, Gulbostan Ismayil & A. Abbas 20111143 (XJU).

Comments — When first collected in the field, *A. volcanica* was regarded as representing the common *A. cinerea* (type species of the genus), which differs by its gray to almost white thallus with quite large, sometimes confluent apothecia, a brown to olive-brown epihymenium, slightly shorter ascospores (usually <20 µm long), pycnidia and conidia present, and the presence of norstictic acid (Owe-Larsson et al. 2007). *Aspicilia volcanica* is further distinguished by its smaller somewhat ± dispersed areolae, cortex without crystals, smaller apothecia, and larger ascospores.

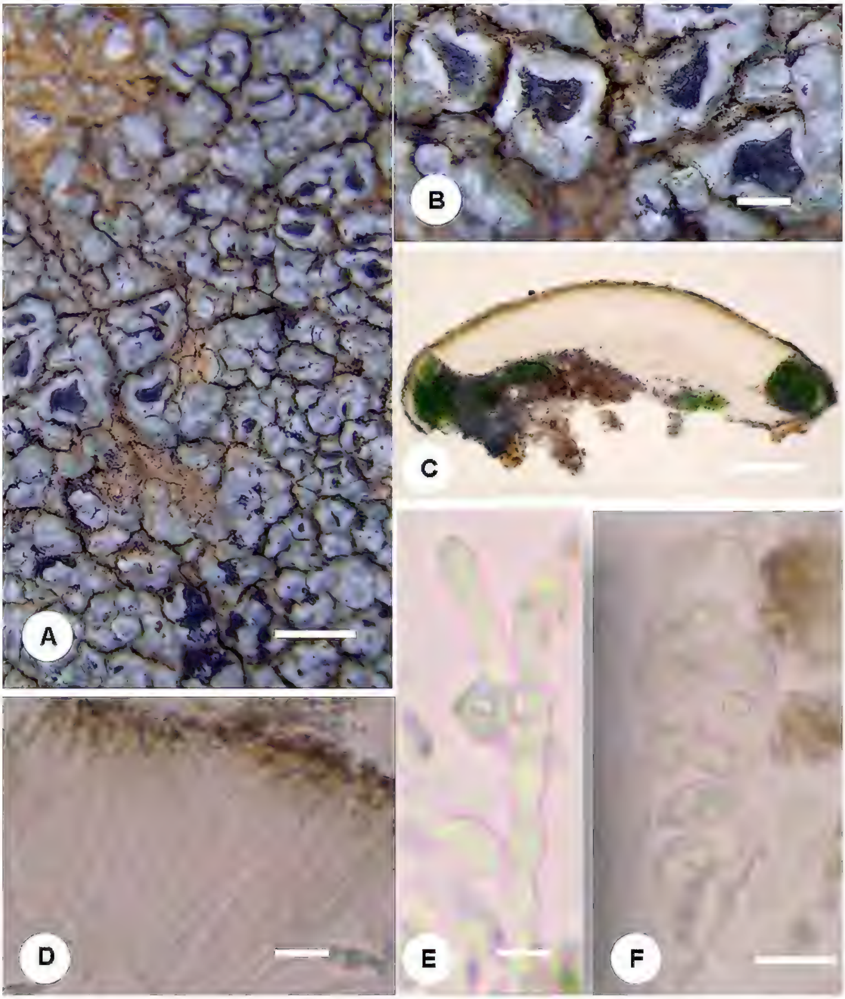

Figure 2. *Aspicilia volcanica* (holotype). A: general habit; B: Apothecia; C: Section of Apothecium; D: Anastomosing paraphyses; E: Moniliform paraphysis; F: Asci and ascospores. Scale bars: A = 3 mm; B = 1 mm; C = 200 µm; D = 25 µm; E = 10 µm; F = 20 µm.

Previous studies that have used DNA sequence data for species recognition in lichens required both diagnostic morphological differences and genetic distance in single-locus nrITS sequence (e.g., Nordin et al. 2011, Han et al. 2013). Our morphological and molecular data for *A. volcanica* match these criteria.

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